ELECTROPHORETIC STUDIES OF SERUM PROTEINS AND GLYCOPROTEINS IN ACUTE CANINE DISTEMPER

by

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INTRODUCTION

Utilization of erythrocyte and leucocyte counts and erythrocyte sedimentation rates in disease study and diagnosis is well known. The cellular elements of the blood, because of their size, have been extensively studied since the advent of good microscopes. Studies on the blood proteins, which contribute to the buffering action, transportation of enzymes, minerals, lipids, amino acids, sugar, vitamins and other nutrients, the clotting mechanism, antibody protection, and water balance lagged for a long time. Older methods available for studying the humoral portion of the blood were complex. The recent development of practical electrophoretic equipment has provided a relatively easy method for the separation of serum proteins. Knowing what serum protein changes occur in certain pathological conditions is of value, both as an aid to diagnosis, and to the better understanding of certain disease processes.

REVIEW OF THE LITERATURE

History of Electrophoresis

Electrophoresis is defined as "the migration of charged colloidal particles through the medium in which they are dispersed, when placed under the influence of an applied electrical potential" (Jones, Hoerr, Osal, 1951). The earliest studies concerned with the separation of dissolved substances by electrical current were made by Hittorf (1853) and Oliver Lodge (1866). Hardy (1899), the founder of colloid chemistry, termed the process "cataphoresis" and Michaelis (1909) referred to it as "electrophoresis". Tiselius, in 1925, continued the studies begun by Svedberg and Scott (1924) and in 1937 (Tiselius, 1937a) published the design of the first practical apparatus for the separation of protein. This instrument consisted of a U-tube for the separation of a mixture into individual fractions and an optical system for observing and recording the boundaries between these colorless solutions. This method of separation has been referred to as "boundary" or "free" electrophoresis, for at the end of the experiment the components of the mixture are only partially separated (Longsworth, 1959, p. 94), and only the fastest and slowest moving fractions can be removed from the U-tube for further analysis. There were two other disadvantages of the Tiselius apparatus. Prior to electrophoresis 5 ml. of serum had to be diluted to 10 to 15 ml. with buffer and dialized at 0 to 6° C. against two 250 ml. portions of buffer for one day each, and then for a third day against sufficient buffer (1500 ml.) to fill the apparatus. The serum was then strongly centrifuged to make it clear and avoid optical error when the separation was evaluated. The water bath required to maintain a constant temperature to prevent convection currents and the optical system used to evaluate the separation made the apparatus large and complex (Longsworth, 1959, p. 163).

The next major advance in the field of electrophoresis was the development of a process that would permit separation of components to the degree that each zone would be separated from the next by a clear space, and which would permit isolation of each fraction. This was called zone electrophoresis. (Tiselius, 1959). Von Klobusitzky and Koni (1939) reported using paper strips saturated with an electrolyte solution to separate a yellow pigment from snake venom and to separate a mixture of dyes. Haugaard and Kroner (1948), Wieland (1948), Wieland and Fischer (1948) and Biserte (1950) reported the separation, with the aid of direct current, of amino acids on filter paper saturated with a buffer solution. Cremer and Tiselius (1950), Durrum (1950), Kraus and Smith (1950), McDonald et al. (1950), Turba and Enenkel (1950) and Grassmann and Hannig (1950) described the separation of macromolecules, mostly proteins, by filter paper electrophoresis.

Principles of Electrophoresis

Proteins have been demonstrated to be amphoteric compounds, each of which has its own isolectric point. This property enables them to be separated in an electrical field. An amphoteric compound has been described as one which can act either as an acid or a base. To state this in another way, the molecule carries both positive and negative charges--Zwitter ions. The positive charge results from the ionization of the amino group ($-NH_3^+ + -OH^-$) and the negative charge from the ionization of the carboxyl group ($-COO^- + H^+$) on the peripheral amino acids of the protein molecule. At normal body pH the negative charges outnumber the positive, thus giving an overall negative charge to the protein molecule.(Wuhrmann and Wunderly, 1960, p. 94).

The isolectric point has been defined as that pH at which the molecule carries no overall electrical charge (Edsall, 1943). This state was first observed by Hardy (1905). He observed that for each protein there was a definite pH at which it became unstable. He also noted that globulins and casein flocculated, while albumin, hemoglobin and gelatin became less dispersed. This effect of pH on protein dispersion resulted from the fact that when the hydrogen ion concentration was increased, there were more hydrogen ions present to react with the $-COO^-$ groups, thus reducing the number of free $-COO^-$ radicals present at any one time and in turn reducing the total negative charge on the molecule. When the number of negative charges equaled the number of positive charges, the repelling force was abolished and they aggregated. Since at this pH there was no net electrical charge, the molecule would also remain stationary if the solution were placed in an electrical field. If the hydrogen ion concentration were further increased, the protein molecules acquired a positive charge for the number of $-NH_3^+$ groups would exceed the $-COO^-$ group and dispersion recurred. On the other hand, if hydroxyl ions were added when the protein was negatively charged, there was an increase in the number of ionized carboxylic groups, and, thus, an increase in overall negative charge.

The isolectric points for the various serum protein fractions are: albumin 4.64, alpha globulin 5.06, beta globulin 5.12 and gamma globulin 6.0 (Tiselius, 1937b). In view of the relationship between isolectric point, pH and electrical charge, at an alkaline pH albumin moved the fastest and gamma globulin the slowest. The rate of migration could be varied by altering the charge on the molecule, temperature and/or viscosity of the media or the voltage of the electrical field (Wuhrmann and Wunderly, 1960, p. 98).

In performing electrophoresis several factors were considered when selecting the buffer solution. The first, and probably most important, was pH. The pH selected was that at which the mixture of proteins to be separated was stable, and which allowed sufficient charge to develop on the protein molecule to permit a satisfactory separation when an electrical current was passed through the mixture. A second requirement was that the pH of the buffer remain constant during the electrophoretic run. The third consideration was the chemical composition of the buffer system. Buffer ions were adsorbed onto the proteins and thus contributed to the charge and altered their isolectric point. Both the number of ions adsorbed and their ionic valence were important in this respect. Monovalent buffer systems were preferred. The diethylbarbituric acid buffer system at a pH of 8.6 was the system most frequently used in paper electrophoresis. This system provided both large buffer capacity and low ionic strength (Wuhrmann and Wunderly, 1960, p. 97).

The principles stated above for the separation of serum proteins applied also to the electrophoretic separation of glycoproteins, lipids and lipoproteins. The main difference in technique for each of these was the employment of a stain that was selective for the desired chemical group. Proteins were most commonly stained with amido black 10B and bromphenol blue. The use of acid wool dye azocarmine B, neococcineacilan scarlet, Ponceau 2R and light green S.F. were also proposed. Mucoproteins were demonstrated with the periodic acid Schiff reagent. Lipids and lipoproteins have been stained with Sudan black B (National Aniline), oil blue N (National Aniline), Sudan III, lipid red 7B (Ciba), Sudan IV and oil red O. The most critical requirement for all of these stains was that they combine with their respective compounds in direct proportion to the concentration of the substance (Wunderly, 1959, p. 203.

Evaluation of the stained strips was done in three ways: elution method, reflection method, or by direct photometry. In the elution method the stained and dried strip was cut transversely into 5 millimeter wide rectangles. Each of these rectangles was then placed in a test tube with the appropriate solvent for elution. The unstained pieces provided the blank value. These had to be determined each time because of variations in the stain vat and rinsing procedure. When the paper strips became colorless, the supernatant was poured off and the optical density determined in a photoelectric cell equipped with a filter appropriate for the dye. If the original strip had been serially cut and eluted, plotting the values of optical densities on the ordinate and distance of the strip from the origin on the abscissa produced a gradient curve comparable with that obtained by free electrophoresis. In clinical determinations, where a gradient curve was not necessary individual fractions were cut out in toto and eluted (Wunderly, 1959, p. 208).

The reflection method of paper strip analysis was accomplished by measuring the decrease in the intensity of light reflected from the stained areas. Owen (1956) described the method in detail. This method has received little application.

In direct photometry light was passed through the stained paper strip and the resultant variations in light intensity caused by the stained material were measured by a photoelectric cell and galvanometer. The instruments available today employ light filters to provide monochromatic light for determining these differences in optical density, and also provide a selection of filters to be used with various dyes. The use of monochromatic light instead of white light was found to be more accurate, for with monochromatic light there was a linear relationship between the optical density and actual amount of dye present. These instruments ("Elphor-Integraphy" , Bendir and Hobein, Munich 15, Germany; "Analytrol" SpincoModel RB, Spinco Division, Beckman Instruments, Inc., Belmont, California) have been found to be advantageous for they automatically record the variations in optical density as a curved tracing on millimeter lined paper, and through an integrator record the area circumscribed by the curve. The percentage of each individual protein could then be determined by dividing the area circumscribed by a particular fraction by the total area of the mixture (Wunderly, 1959, p. 211).

Serum Proteins

Tiselius, in his first electrophoretic studies of human serum, obtained four fractions. The fraction of the highest concentration and fastest mobility was identified as albumin, the other three were arbitrarily named alpha, beta and gamma in order of decreasing mobility (Moore, 1959).

Albumin. Albumin has been determined to constitute 38-53 per cent of the total protein in animal sera and about 60 per cent of the total protein in human serum. The molecular weight has been determined to be about 66,000, making it the smallest of the principle blood proteins. Despite this uniformity in molecular weight, the broad base obtained in electrophoretic separation indicated that albumin is a heterogeneous mixture of proteins. The properties of high percentage and small molecular weight indicated that it exists in a high concentration, estimated to be 3×10^{17} molecules per milliliter in human serum. Because of this high concentration and the resultant effect on the colloid osmotic pressure of the blood, the primary function attributed to albumin was maintaining fluid balance. Another function of greater importance attributed to albumin than to the other protein fractions was the binding and transporting of substances such as anionic and catatonic dyes, anions of fatty acids and aromatic carboxylic acids, acetylated amino acids, penicillin and sulfomanide derivatives and heparin. Albumin, in contrast to the other proteins, has been found to bind and inactivate certain toxins, metabolic inhibitors and enzymes (Moore, 1959, p. 379). The liver has been thought to be the main site of albumin synthesis. Evidence to support this theory was seen in cases of severe liver disease where there was a marked decrease in albumin, in experiments with liver slices and radioactive substances by Peters and Anfensin (1950) and with perfused whole rat livers by Miller et al. (1951) and Campbell and Stone (1957).

Alpha and Beta Globulins. The alpha fraction of Tiselius has been further subdivided by using buffers of higher alkalinity. Longsworth (1942) used a veronal buffer, pH 8.6, and described a fraction between albumin and alpha which he called alpha 1 globulin. Immunologic processes have demonstrated two alpha 1 and five alpha 2 subfractions (Grabar and Williams, 1959).

The molecular weight of alpha and beta globulins has been determined to range from 90,000 to a million. They have been involved in the transport and exchange of cholesterol, phospholipids, fatty acids and various fat soluble vitamins and hormones. Properdin is a normal constituent of beta globulin. The liver was found to synthesize some of these proteins, but in essence their site of formation has not been determined (Moore, 1959, p. 382).

Gamma Globulin. Gamma globulin was found to be composed of molecules having an approximate gram molecular weight of 160,000 although larger molecules have been demonstrated. Electrophoretic, immunological and chemical techniques have shown this fraction to be very heterogeneous. Gamma globulin has been demonstrated to be primarily concerned with antibody activity, although small amounts of antibodies have been found in all the other fractions. Very extensive work has been performed in trying to determine the site of gamma globulin synthesis. At present the lymphocytes, reticuloendothelial cells and plasma cells are thought to be the primary sites of formation (Moore, 1959, p. 382).

Dysproteinemia in Human Serum

Albumin Alterations. Relative and absolute increases in albumin have rarely been found in human sera. Jorke and Heuchel (1956) reported low grade relative and absolute increases in albumin in convalescent cases of liver disease and subacute bacterial endocarditis. Wuhrmann and Wunderly (1960, p. 423) stated that high normal or slightly elevated albumin values occurred only in hypoglobulinemia or agammaglobulinemia. Hypoalbuminemia, on the other hand, occurred in such conditions as nephrotic syndrome, chronic hepatitis and gastrointestinal diseases (Kessel and Kessel, 1954).

The nephrotic syndrome has been characterized by an electrophoretic pattern showing a marked reduction in albumin, elevated beta and alpha globulins and a normal or decreased gamma globulin. Current publications indicated that this condition resulted from the increased permeability of the glomeruli for blood protein (Chenard et al., 1954; Chaptal et al., 1955; Cavelti, 1946; Sarre, 1953; Moench et al., 1955; Grass, 1956; Burch et al., 1954; Taylor, 1956; Merrill, 1957). Albumin, and to a lesser extent gamma globulin, and only a small amount of beta and alpha 2 globulins were found in the urine (Wuhrmann and Wunderly, 1960, p. 430). Signs of this condition were severe generalized edema, an elevation of blood cholesterol, phospholipids and fatty acids, hypocalcemia, hemoconcentration and decreased resistence to infection (Wuhrmann and Wunderly, 1960, p. 341).

In chronic liver diseases a decrease in total serum protein, mainly albumin, has been found. The degree of diminution usually indicated the severity. The electrophoretic pattern showed, in addition to the decreased albumin, a normal alpha globulin, slight increase in beta globulin and an increase in gamma globulin. According to Emrich and Petzold (1955), the higher the gamma globulin, the more unfavorable the prognosis. The increased gamma fraction was thought to result from the stimulation of the Kupffer cells and histocytes in the liver (Wajchenberg et al., 1956).

Gastrointestinal diseases, especially those associated with persistent and severe diarrhea, have been shown to cause a loss of electrolytes, fluid and protein, mainly albumin, in the feces. Other conditions, such as pancreatogenic diarrhea, chronic enteritis and cancer of the stomach, also produced a hypoalbuminemia (Wuhrmann and Wunderly, 1960, p. 448).

Globulin Alterations. Electrophoretic patterns of sera from individuals with many diseased conditions usually showed an increase in a globulin fraction or fractions and a compensatory decrease in albumin. First impressions have suggested that the albumins were transposed into globulins or that globulins were produced in preference to albumin. Wuhrmann and Wunderly (1960), p. 347, however, stated that this compensatory decrease was the result of the influence of an extremely rigid central regulating mechanism concerned with maintaining an optimum osmotic pressure. They suggested that when the globulin fraction was increased, the liver and spleen removed and stored the excess albumin. Bjorneboe (1945), Whipple (1956) and Grass (1950) presented similar views.

Alpha Globulin. Normal alpha globulin has been shown to contain 1.89 to 4 per cent protein bound polysaccharides--mucoprotein (Wunderly and Peller, 1954; Bergstermann, 1956; Raymaud et al., 1952; Adreani, 1955; Greenspan, 1954; Stary et al., 1953). Puls and Albaum (1956) stated than an increase in alpha 1 globulin was in reality an increase in mucoprotein, and that of the serum proteins, alpha 2 globulin was the richest polysaccharide component.

Alpha hyperglobulinemia has been demonstrated in inflammatory, infectious and necrotic conditions. Frequently there was an associated increase in beta globulin. The degree of alpha globulin increase indicated the acuteness, severity and extent of the inflammation, as well as the response of the body (Bellet, 1959). A severe infection accompanied by a favorable response caused a marked elevation in alpha globulin. On the other hand, Scheurlin (1955) demonstrated that infections with pre-existing inflammation as well as cyclic infectious diseases and inflammations associated with highly developed immunity produced no change or a minimal increase in alpha globulin.

Alpha globulin increases have been observed in conditions producing a negative nitrogen balance (Litterer and Schneider, 1953), eg. neoplasms, metabolic diseases, the nephrotic syndrome and true protein dificiencies (Lewis et al., 1950; Chow, 1947). Zeldes and Alling (1945) demonstrated that alpha 2 globulin increased rapidly following plasmapheresis. Similarly alpha 2 globulin has been observed to rise following removal of a large quantity of blood.

Relative and absolute reductions in alpha globulin have been found rarely. Von Studnitz showed a decrease to occur in hepatic cirrhosis and in hyperchromic and hemolytic anemia.

Beta Globulin. Beta hyperglobulinemia rarely occurred by itself; it was usually accompanied by an increase in alpha 2 globulin. Elevated beta globulin has been observed in chronic liver disease, diabetes mellitus, hypothyroidism, amyloidosis, the nephrotic syndrome, some cases of exudative pulmonary tuberculosis, some skin conditions, xanthomatous cirrhosis, lipoidemia, beta macroglobulinemia, and beta plasmacytoma (Wuhrmann and Wunderly, 1960, p. 353).

Gamma Globulin. Gamma hyperglobulinemia was found in one-third to one-half of the diseases causing hospitalization (Grass, 1956; Soulier, 1955; Wall, 1958). Variations in gamma globulin were considered by Wuhrmann (1950) to be indicative of the body's response, and not of disease activity itself. Gamma globulin level was thus thought to be of prognostic value. If gamma globulin was normal to slightly elevated in a severe chronic disease, the prognosis was unfavorable. Scheurlin (1955) stated that the degree of gamma globulin increase was dependent on constitutional factors and the anamnestic response.

Increases in gamma globulin were subdivided into homogeneous and heterogeneous gamma hyperglobulinemia. Homogeneous gamma hyperglobulinemia occurred only in the gamma type plasmacytoma and macroglobulinemia. The electrophoretic pattern was characterized by a narrow based, pointed, high peak in the gamma globulin fraction (Wuhrmann and Wunderly, 1960, p. 359).

Heterogeneous gamma hyperglobulinemia was characterized electrophoretically by a broad base, rounded peak gamma globulin fraction. This has been reported in immunoserological processes, chronic infectious diseases, chronic liver diseases, especially cirrhosis and chronic hepatitis, and malignant tumors. The increase in gamma globulin associated with malignant neoplasms with wide spread metastases has not been understood except in those cases where the liver is destroyed (Wuhrmann and Wunderly, 1960, p. 359).

Diminution of gamma globulins has been observed in the nephrotic syndrome, rare types of hepatocellular damage and chronic nutritional diseases (Jorke and Heuchel, 1954), early stages of infectious diseases (Scheurlen, 1955), lymphosarcoma (Wall, 1958), amyloidosis and paramyloidosis (Wuhrmann and Wunderly, 1960, p. 366) and agammaglobulinemia (Moore, 1959, p. 406). One significant factor associated with a decrease in gamma globulin was a predisposition to infection.

Canine Serum Proteins

Normal Values. Stockl and Boguth, quoted by Vesselinovitch (1959), summarized the values presented by other authors and gave the following values as normal for the dog: albumin 53.5 per cent; alpha globulin, 13.8 per cent; beta globulin 20.4 per cent; gamma globulin 12.3 per cent.

Dysproteinemia. Gjessing and Chanutin (1946) showed the effect of non-infectious inflammatory conditions on serum proteins using various irritants. In their first experiment they exposed all but the head of the dog to a high concentration of sulfur mustard vapor (500 gamma per liter of beta chloroethyline vesicant vapor) for 28 minutes. Blood was drawn prior to exposure and just prior to death. By comparing the area covered by the protein fractions on an electrophoretic graph, they estimated that in the second sample, albumin decreased 50 per cent and alpha globulin increased 350 per cent. In their second experiment, dogs that died following injection of the vesicant also exhibited the same alpha globulin-albumin alteration. In order to study the effects of other inflammations, these authors performed the following series of experiments. They anesthetized a dog, clipped the hair off the whole body, except for the shoulders and head, and then immersed the clipped portions of the animal into 73° C. water for 6 seconds. At the end of the first 24 hours the albumin decreased from 3.31 to 1.31 gm. per cent and the alpha globulin increased from 0.29 to 1.03 gm. per cent. By the third day albumin had increased to 1.89 gm. per cent and alpha globulin increased to 1.89 gm. per cent. On the fourth day albumin had increased to 2.0 gm. per cent and was maintained. Alpha globulin continued to increase and constituted 2.0 gm. per cent of the serum when the animal was euthanatized on the seventh day because of large areas of skin necrosis. Next an anesthetized dog was injured to the same extent as the above, but dry ice instead of heat was used. This animal also showed a decrease in albumin and a 240 per cent rise in alpha globulin. They also observed that injections of 0.5 ml. of turpentine subcutaneously into six sites on a dog's back produced a decrease in albumin and increase in alpha globulin with the most marked alteration occurring the third day following the injections. Aseptic fractures of the tibia in anesthetized dogs produced by the use of an osteotome did not produce any alteration in albumin during the 17 day observation period, but did cause an increase in alpha 1 and alpha 2 globulins with the most marked increase occurring on the third day. Beta and gamma globulins remained normal. They next squeezed about 120 sq. cm. of abdominal skin of an anesthetized dog by use of clamps and a vise. This produced no serum alterations during the four day observation period.

Keil (1954) observed that the sera from canine surgical cases first showed an increase in alpha globulin and later an increase in gamma globulin. There was a compensatory decrease in albumin.

Alterations of canine serum proteins in infectious diseases have been reported by several individuals. Chabaud et al. (1955) reported a marked increase in alpha globulin in rabid dogs. They considered this alteration specific and stated that it might be of value for early diagnosis of rabies in a living animal. Polson and Malherbe (1952), using free boundary electrophoresis, demonstrated the following changes in the various forms of Babesia canis infection: peracute form, albumin 1 and 2 decreased, alpha globulin increased, beta globulin remained normal and gamma globulin slightly increased; cerebral form, albumin 1 and 2 decreased, alpha globulin markedly increased, beta 1 and beta 2 remained normal and gamma globulin increased; the acute form before treatment, albumin 1 decreased, albumin 2 slightly decreased, alpha 1 increased, alpha 2 slightly increased and beta and gamma globulins remained normal. Sera at five and again at ten days post treatment had only an increased gamma globulin. Sera from five dogs with rickettsiosis showed a rapid decrease in albumin 1, little change in albumin 2, alpha 1 globulin somewhat increased, alpha 2 globulin considerably increased, beta 1 globulin greatly increased, beta 2 globulin less increased and gamma globulin moderately increased. Polson and Malherbe (1952) reported that sera from dogs with respiratory, gastrointestinal, nervous or hard pad forms of distemper showed no particular deviation in electrophoretic pattern except for a decreased albumin due to emaciation in serum taken in the advanced stages of the disease. Gentile et al., (1960) found in distemper an increase in alpha 2 and gamma globulins and a decrease in albumin.

Campbell (1957) stated that he was able to confirm Boguth's (1954) finding that a closed pyometra was characterized by an increase in beta globulin. In addition, Campbell (1957) noted that sera from several discharging pyometras showed increased alpha globulin and a normal or decreased beta globulin. Boguth (1954) claimed, according to Campbell (1957), that a commitant rise in alpha globulin in pyometra denoted peritonitis and a poor prognosis. In eosinophilic myosites and chronic skin diseases, Boguth (1954) found an excessive increase in beta globulin. Ebel (1953) noted either a slight increase or decrease in albumin and a rise in alpha 2 and beta globulins in the sera from nine dogs with experimentally induced canine infectious hepatitis. Boguth (1954) stated that leptospirosis may be associated with an increase in both beta and gamma globulins or only in beta or gamma globulins. Gentile et al. (1960) reported an increase in alpha 2, beta and gamma globulins in leptospirosis.

Archibald and Vesselinovitch (1957) produced hepatic fatty degeneration and necrosis by ligating the hepatic artery. The mean values from sera of three dogs, collected before surgery and at 24, 48, 72 and 118 hours post surgery, showed a decrease in albumin from 43.1 to 21.1 per cent, an increase in alpha globulin from 19.9 to 37.6 per cent, an increase in beta globulin from 18.7 to 25.7 per cent and a decrease in gamma globulin from 18.3 to 15.6 per cent. At no time did a hypoproteinemia develop. Campbell (1957) reported a marked increase in gamma globulin and decrease in albumin in hepatic cirrhosis. Gentile et al (1960) found a decrease in albumin and increase in beta and gamma globulins in hepatic cirrhosis. De Wael (1956) claimed that hepatitis and cirrhosis could be differentiated electrophoretically. In hepatitis there is a pronounced alpha 2 globulin band; in cirrhosis the beta and gamma globulins are markedly increased and poorly resolved.

Moegle et al. (1956) analysed the sera from 63 dogs with kidney disease and found the typical nephrotic syndrome changes of decreased albumin and gamma globulin and increased alpha 2 and beta globulins only in severe cases. Campbell (1957) on the other hand, found in cases of chronic nephritis, a decrease in albumin and an increase in alpha and beta globulins. When edema was present gamma globulin was also increased. He attributed this rise to liver involvement. Stickler et al. (1956) produced nephrosis in nine dogs by injecting them with nephrotoxic serum produced in rabbits. The first day after injection they observed proteinuria and then a hypoproteinemia characterized by a decrease in albumin and gamma globulin and an increase in alpha 2 and beta globulins. The increase in alpha 2 globulin was marked. Eight to 12 days after injection the severity of the proteinuria diminished and by the fortieth day post injection five of the nine dogs were normal.

Bossay et al. (1955) reported a selective elevation of alpha 2 globulin to twice normal in dogs receiving cortisone. There was an associated decrease in beta globulin, but no hypoalbuminemia or decrease in total protein.

Boguth (1954) made the following generalizations on the alterations in serum protein: acute inflammation, particularly in dog and swine, was associated with an increase in alpha globulin; chronic and subacute conditions (polyarthritis, various infectious diseases and chronic nephritis) were characterized by an increase in gamma and beta globulins, and malignant diseases show an increase in alpha, beta and gamma globulins. In all three of the above, albumin was decreased.

Glycoproteins

Freund (1892) was the first to isolate a heat stable protein-carbohydrate complex which was soluble in strong acid protein precipitating agents. Morner (1893), Zanetti (1897) and Bywater (1909) reported a similar substance. These workers named the compound "seromucoid". Subsequently others studied protein-carbohydrate substances from various sources, using different methods for isolation and carbohydrate measurement, and referred to the compounds as index of polypeptidemia, polarigraphic filtrate wave, blood proteose, seroglycoid, serum glycoprotein, acid glycoprotein, mucopolysaccharide, globoglycoid and fraction IV mucoprotein. Winzler (1960), p. 311, attempted to rectify this confused state of terminology by using glycoprotein to indicate those components which have primarily a protein character and which contain appreciable (greater than 1 per cent) amounts of carbohydrates bound in such a manner that vigorous hydrolysis is required to liberate it. Winzler (1960), p. 311, stated that a better designation might be those proteins having at least one mole of hexose or hexosamine per mole of protein. He used the terms proteinaminopolysaccharide or mucoprotein for protein and acid aminopolysaccharide complexes. This complex could be hydrolyzed by treatment with relatively mild alkali.

The amount of protein-bound carbohydrate in normal human plasma has been shown to exceed considerably the free glucose level (272 mgm./100 ml.). Six monosaccharide derivatives have been recognized as components of glycoprotein: galatose, manose, glucosamine, galactosamine, fucose and sialic acid (Winzler, 1960, p. 310).

Glycoproteins have been separated by electrophoresis. The major fractions resulting from electrophoretic separation were referred to as tryptophane-rich pre-albumin, alpha 1, alpha 2, beta and gamma glycoproteins.

Tryptophane-rich pre-albumin moved 50 per cent faster at a pH 8.6 than albumin and contained 2.5 to 2.7 per cent tryptophane. Its concentration in normal serum was found to be very low and thus contributed little to the total protein bound carbohydrate. The only demonstrated activity of the fraction was binding of thyroxine (Winzler, 1960, p. 316);

The alpha 1 glycoproteins were found to be relatively high in carbohydrate and to contain about 7.5 per cent hexose in comparison to 1.6 per cent hexose for whole serum. In pathological conditions the percentage of carbohydrate has been shown to rise. This fraction has been subdivided into several glycoproteins: orosmucoid, a viral hemagglutination inhibitor; a nonspecific hyaluronidase inhibitor; and a trypsin inhibitor. Of these orosmucoid has been most studied. Its significance has not been demonstrated, but it has been reported to increase clotting time by inhibiting the conversion of prothrombin to thrombin, and to influence the spacing of collogen fibers (Winzler, 1960, p. 317).

The alpha 2 glycoproteins have been shown to possess the highest carbohydrate content. This fraction was found to contain several glycoproteins: cerulophosmin, which transports 90 per cent of serum copper (Holmberg and Laurell, 1948); haptoglobins, which bind hemoglobin released from erythrocyte to form a high molecular weight compound and thus prevent excessive loss of iron in the urine (Laurell, 1960, p. 360); alpha 2 macroglobulins; several low molecular weight glycoproteins and prothrombin (Winzler, 1960, p. 321).

The beta glycoproteins have been ranked second to the alpha 1 glycoproteins in carbohydrate content. Two subfractions, transferin and beta 2A globulin, have been studied (Winzler, 1960, p. 325). Transferin is involved in iron transport (Laurell, 1960, p. 354).

The gamma glycoproteins also were found to contain considerable carbohydrate (Porter, 1960). These were separated into large and small fractions by zone electrophoresis and by ultracentrifugation. The smaller fraction was estimated to have a molecular weight of about 160,000 and a carbohydrate content of 3.1 per cent, while the larger fraction was estimated to have a molecular weight of about 1,000,000 and a carbohydrate content of 10.4 per cent (Winzler, 1960, p. 326).

In humans, serum mucoprotein (glycoprotein) was increased in over 90 per cent of patients affected with acute infectious diseases such as pneumonia, enteritis, cellulitis, tuberculosis and brucellosis. Subacute or chronic conditions such as endocarditis, ileitis, colitis, sinusitis, diverticulitis, bronchiectasis and tuberculosis, produced an increase in 75 per cent of the cases, with the elevations occurring primarily in the more active cases. Renal diseases, as acute glomerular nephritis and the terminal uremic stages of glomerular nephritis, pyelonephritis and nephrosclerosis, also increased serum glycoproteins. Increased levels were found in over 80 per cent of patients with rheumatic fever; the degree of increase indicated the severity and correlated well with erythrocyte sedimentation rates and clinical symptoms. Serum glycoproteins were also elevated after myocardial infarction, congestive heart failure, trauma, surgery, and irradiation (Greenspan, 1955). Kushner et al. (1956) administered ACTH to humans and noted an immediate rise in serum glycoproteins. The increase was clearly related to dosage. Two 30 to 40 unit dosages on consecutive days produced a maximum response. Decreases in glycoprotein level, on the other hand, have been seen in hepatocellular diseases such as infectious hepatitis, homologus serum hepatitis, toxic hepatitis and portal cirrhosis and in failures of the pituitary and adrenal glands (Greenspan, 1955).

In contrast to the numerous investigations on glycoprotein alterations in humans, the number of reports concerned with glycoprotein levels in domestic animals was limited.

Shuttar et al. (1949) investigated the effect of various inflammatory agents on serum polysaccharide levels. In one experiment they injected subcutaneously 5 ml. of a mixed culture of *Staphylococcus aureus* and *Streptococcus pyogenes* into the rear legs of healthy dogs. An abcess 10 cm. in diameter formed, ruptured spontaneously, and drained on the sixth day; the skin sloughed on the eighth day and the lesion was healed by about the sixtieth day. The temperature was highest on the third day following injection and returned to normal on the sixth day. The non-glucoseamine polysaccharide level, expressed as per cent protein, on zero day was 2 per cent, on the first day it was slightly elevated and on the sixth day it reached a maximum of about 4.2 per cent and then gradually decreased and became normal on the fifty-second day. Turpentine injected intramuscularly or intrapleurally produced a similar response. Injection of 20 gm. of talc in 80 ml. of water produced signs of peritoneal irritation for three hours, no appreciable temperature rise, but an extensive granuloma resulted. The serum polysaccharide level reached a maximum of 3.2 per cent on the third or fourth day and remained elevated on the twentieth day. An increase of serum polysaccharide from a normal of about 2 per cent to about 2.6 per cent resulted from laparotomy.

Tradati and Abbate (1958) analysed serum from 33 dogs with acute and chronic skin conditions and found the mucoprotein fraction was elevated from a normal of 1.82 mg. of tyrosine per 100 ml. to 2.25 mg. and 1.90 mg. respectively.

Zecha, Kalousova and Kucima (1959) reported that the sera from dogs irradiated with 600 r showed a significant elevation of values of the globulins and glycoproteins. These changes began the second day after irradiation and attained maximum levels just prior to death.

There have been theories proposed for the origin or source of the increased amounts of glycoproteins. Gersh and Catchpole (1949) proposed that the increase might arise from a connective tissue (glycoprotein) component. Following trauma and also following induced lung edema, they demonstrated that the ground substance of the connective tissue in the affected area changed and became water soluble. They believed this change was produced by depolymerizing enzymes secreted in the area that broke down the complex insoluble ground substance to simpler soluble substances. These diffused into the blood stream and appeared as glycoprotein. Catchpole (1950) presented more evidence to support this theory. Shetlar et al. (1949) concluded that since the increase in serum polysaccharide was delayed 3 to 4 days it was not correlated with the degree of tissue destruction and was not dependent on fever, and asserted that the elevation must result from tissue proliferation and repair.

In view of these theories, experimental observations and clinical observations, serum glycoproteins were included in the "acute phase" reactants. The degree of elevation was found to be regulated to a great degree by the general metabolic factors in animals and to be particularly dependent on the liver, hypophysis and adrenal glands. The initial increase that occurred within a few hours after stress was thought to result from adrenal-cortical response, and the subsequent increases which were of greater magnitude and persistence, were probably due to tissue injury and repair. (Kusher et al., 1956).

MATERIALS AND METHODS

The 20 dogs in groups I through VI were received on May 21 from southwestern Kansas. The dogs were bled on May 23 and blood for total leukocyte count and serum was collected. On May 25 the serum neutralization test was performed on the sera. May 26 the dogs were divided into groups 1 through 5. Each of these groups was handled as indicated in table 1. On the day of challenge and on every other day subsequently, the dogs were bled and blood for leukocyte count and serum was collected. The outcome, death or survival of the dog, in this portion of the experiment governed the formation of electrophoretic groups I through VI. The composition of these groups is stated in table 2.

Table 1. The original grouping, major procedures performed and results for the dogs received on May 21.

Group	No. of dogs	SN titer on May 23 serum	Date vaccinated	Date challenged	Outcome
1	4	less than 1:20*	May 26	May 31	2 died
2	4	less than 1:20	May 27	May 31	4 died
3	4	less than 1:20	May 28	May 31	3 died
4	4	less than 1:20*	May 29	May 31	3 died
5	5	less than 1:20	not vac.	May 31	5 died

* 1 dog had a 1:40 titer.

Table 2. Electrophoretic grouping of dogs received May 21.

Group:	Dogs composing the group.
I	2 dogs of original group 1 that died
II	3 dogs of original group 2 that died
III	3 dogs of original group 3 that died
IV	3 dogs of original group 4 that died
V	5 dogs of original group 5 that died
VI	2 dogs with titers plus 1 from group 1 and 1 from group 3

Electrophoretic groups VII and VIII consisted of 8 dogs selected from a group of 29 dogs received from Arkansas on April 19. Up to May 11, the day of challenge, these dogs were handled in essentially the same manner as the dogs described above. Many of the dogs had a harsh cough on the day of challenge. Starting May 16 the dogs were bled every other day through May 24 for both leukocyte count and serum. On May 25 the serum neutralization test was performed on the control dogs and a few others selected at random, using serum collected on May 24. After May 24 serum was collected when the animal was moribund. The important information concerning the dogs in groups VII and VIII is listed in table 3.

Table 3. Important procedures performed on dogs in electrophoretic groups VII and VIII.

Group	No. of dogs	SN titer of April 25 serum	Days vac. prior to challenge	Date challenged	SN titer on May 24	Outcome
VII	4	Less than 1:20	not vac.	May 11	greater than 1:40	survived
VIII	4	Less than 1:20	1 dog 4 days 2 dogs 1 day 1 dog not vac.	May 11	less than 1:20	died

The dogs in groups I through V and group VIII died either a natural death or were euthanatized with magnesium sulfate and were bled out when moribund. All dogs were necropsied and blocks of tissue preserved in 10 per cent buffered formalin. Tissue sections were cut at 5 microns and stained with hematoxalin and eosin. The sections from 4 dogs selected at random from groups I through IV were stained with Shorr's S-3 stain.

Serum for the serum neutralization test was inactivated at 56°C. for 30 minutes and then 1:20 and 1:40 dilutions were made with buffered phosphate saline. One ml. of a canine tissue origin virus* containing approximately 200 egg infectious doses--fifty per cent (EID_{50}) was mixed with one ml. of diluted serum and incubated in ice water for two hours. Two tenths ml. from each dilution was inoculated onto the chorioallantoic membrane of 4 embryonated eggs. The eggs were incubated seven days and examined. Two or more plaques constituted a positive membrane.

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* Cytogen, Jensen-Salsbery Lab., Inc.

Leukocyte counts were done on blood in which clotting had been inhibited by dipotassium ethylenediamine tetra acetate (EDTA)*. The counts were performed with a Coulter Counter** using a dilution of 40 lamda of blood in 10 ml. of triple filtered isotonic saline solution and an instrument aperture current (APC) setting of 12 and threshold of 4. The dilutions for groups I through VI were made with an automatic diluter and those for groups VII and VIII were made manually with pipets. The erythrocytes were hemolized by adding 0.1 ml. of a triple filtered solution of 100 mg. of saponin in 10 ml. of isotonic saline. The actual count was obtained by dividing the total count in half and correcting this figure using the table supplied with the Coulter Counter.

The dogs were anesthetized with pentobarbital sodium and challenged intracerebrally with 0.5 ml. of a 1:10 canine brain suspension in buffered phosphate saline. The challenging material was prepared from a dog infected intracerebrally with the Snyder Hill strain of distemper.***

Serum protein separations were performed twice approximately 2 weeks apart on a Spinco Model R Paper Electrophoresis System.**** This system consists of a Durrum-type electrophoresis cell, a model RD-2 Duostat (a regulated power supply for the cell), and a Model RB Analytrol (a calibrated recording densitometer and automatic integrator). Serum protein separation was performed using Spinco B-2 Buffer, Veronal, pH 8.6, package form (2.76 gm. diethylbarbiturate 0.075 ionic strength), Schleicher and Schuell 2043-A mgl. paper strips 3 cm. wide by 30.6 cm. long, 0.006 ml. of serum and a current of 15 milliamps per cell for 5 hours. The connector plugs were reversed (poles reversed) 5 minutes before the strips were removed. The paper strips were dried in a pre-heated oven at 120-130°C. for 30 minutes and then stored in a desiccator charged with calcium chloride overnight. Strips were stained using bromphenol blue dye in alcoholic solution (Spinco Procedure B, technical bulletin No. TB 6050 A) and analysed on the Analytrol equipped with a B-5 cam and 550 millimicron interference filters and using a 1.5 mm. slit width.

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* Cambridge Chemical Products, Inc., Dearborn, Michigan

** Coulter Electronics, Chicago, Illinois

*** Obtained from Jensen-Salsbery Lab., Inc.

**** Spinco Division, Beckman Instruments, Inc., Belmont, California

Serum glycoproteins were separated using the same buffer, Whatman 3 mm. paper strips 3 cm. wide by 30.6 cm. long, 0.03 ml. of serum and a current of 20 milliamps per cell for 6 hours. One separation was performed on each serum sample. Poles were reversed 5 minutes before removing the strips from the cells. The paper strips were dried and stored in the same manner as for the serum protein analysis. The strips were stained with periodic acid-Schiff reagent (Spinco technical bulletin No. TB 6046 B) and analysed on the Analytrol equipped with a B-5 cam, a 500 millimicron interference filter in the front filter holder, a 500 millimicron interference filter and a 0.9 neutral density filter in the rear filter holder and a 1.5 mm. slit width. Because of the development of a white precipitate and loss of staining intensity, new periodic acid-Schiff reagent was needed after staining 32 strips.

RESULTS

The dividing of the dogs into groups and the vaccinating of each group on different days prior to challenging complicated the presentation and analysis of the values obtained. Because of the possibility of vaccination having caused alterations in the serum protein and glycoprotein values in those dogs experimentally exposed to an encephaletic form of distemper, the serum protein and glycoprotein separation results were compared on a group, between groups and overall basis.

Serum Protein, Groups I-VI

The results of the serum protein separations for each group are listed in table 4. The day, number of dogs on which the values given were based, the average value for each serum protein fraction are presented. The terminal values were derived by averaging the figures from the last blood samples drawn prior to death. The primary alterations occurred in the alpha 2 globulin and albumin of groups I through V. Alpha 2 globulin increased slightly on the second, moderately on the fourth and markedly on the sixth and subsequent post challenge days. The terminal alpha 2 globulin values increased markedly. Albumin decreased as the alpha 2 globulin increased. In group VI a small increase occurred in gamma and alpha 2 globulins and a small decrease in albumin. These alterations in the immune group appeared to be maximum between the sixth and eighth post challenge days.

The average serum protein fraction values for each group are compared on a day basis in table 5. The same alterations as observed in table 4 were noted. The significant factor in this table was that vaccination did not influence the serum protein values. Gamma globulin in the immune

Table 4. Comparison of serum protein variations within groups.

Group	Date	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin
I	23 May	2	14.0	4.3	14.2	4.2	67.5
	*31	2	12.6	5.8	10.0	4.5	66.4
	2 June	2	13.4	9.4	12.5	5.1	59.3
	4	2	14.3	6.9	17.5	5.6	55.3
	6	2	13.8	6.0	18.1	4.2	58.7
	8	1	10.2	4.6	23.5	3.0	58.6
	Terminal	2	12.9	5.8	19.2	3.8	59.1
II	23 May	3	15.0	5.7	8.0	6.3	64.1
	*31	3	15.1	5.7	8.9	7.3	59.3
	2 June	3	14.1	7.3	11.0	6.1	61.2
	4	3	15.3	8.4	13.3	5.5	57.4
	6	3	14.7	9.8	19.2	6.1	50.1
	8	1	12.0	7.1	13.3	6.0	61.9
	10	1	13.1	7.2	12.0	5.9	59.7
	Terminal	2	15.4	10.4	21.9	5.7	46.5
III	23 May	3	14.8	4.6	9.1	7.0	64.1
	*31	3	14.2	3.0	10.7	5.8	63.2
	2 June	3	14.8	7.0	11.0	5.4	61.3
	4	3	13.9	7.7	14.1	5.8	53.4
	6	2	14.3	5.2	23.4	4.6	52.4
	8	2	10.1	6.0	26.5	3.3	51.3
	Terminal	3	12.1	5.8	26.4	4.1	49.8
IV	23 May	3	14.9	4.5	10.5	5.1	64.9
	*31	3	15.1	4.5	10.5	6.0	57.9
	2 June	3	19.3	7.4	14.8	5.7	52.7
	4	3	17.1	8.3	17.5	6.3	50.7
	6	3	16.2	8.2	19.1	5.7	50.8
	8	1	13.5	8.4	19.4	5.6	52.8
	Terminal	3	15.8	8.2	19.2	5.5	51.0
V	23 May	5	13.5	4.9	6.2	4.8	68.4
	*31	4	11.8	5.6	9.4	4.8	64.8
	2 June	5	10.9	8.0	9.1	6.0	63.0
	4	5	11.5	6.2	11.8	5.8	61.8
	6	4	11.1	6.9	17.1	6.1	55.9
	8	2	14.8	7.8	19.2	6.0	51.8
	10	1	9.5	6.0	19.9	5.3	59.0
	Terminal	5	10.1	6.5	17.0	5.1	59.0
VI	23 May	4	14.7	7.1	8.1	6.1	64.7
	*31	4	13.8	7.5	10.0	4.9	63.4
	2 June	3	15.4	8.2	10.8	6.2	59.7
	4	4	13.5	9.4	11.7	5.2	60.3
	6	4	16.5	7.5	12.2	5.8	58.1
	8	4	17.3	8.6	11.0	5.2	57.4
	10	4	15.5	5.6	10.8	5.3	62.4

* Day challenged.

Table 5. Comparison of serum protein alterations between groups on a day basis.

Date	Group	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin
23 May	I	2	14.0	4.3	14.2	4.2	67.5
	II	3	15.0	5.7	8.0	6.3	64.1
	III	3	14.8	4.6	9.1	7.0	64.1
	IV	3	14.9	4.5	10.5	5.1	64.9
	V	5	13.5	4.9	6.2	4.8	68.4
	VI	4	14.7	7.1	8.1	6.1	64.7
*31 May	I	2	12.6	5.8	10.0	4.5	66.4
	II	3	15.1	5.7	10.8	7.3	59.3
	III	3	14.2	3.0	10.7	5.8	63.2
	IV	3	15.1	4.5	10.5	6.0	57.9
	V	4	11.8	5.6	9.4	4.8	64.8
	VI	4	13.8	7.8	10.0	4.9	63.4
2 June	I	2	13.4	9.4	12.5	5.1	59.3
	II	3	14.1	7.3	11.0	6.1	61.2
	III	3	14.8	7.0	11.0	5.4	61.3
	IV	3	19.3	7.4	14.8	5.7	52.7
	V	5	10.9	8.0	9.1	6.0	63.0
	VI	3	15.4	8.2	10.8	6.2	59.7
4 June	I	2	14.3	6.9	17.5	5.6	55.3
	II	3	15.3	8.4	13.3	5.5	57.4
	III	3	13.9	7.7	14.1	5.8	53.4
	IV	3	17.1	8.3	17.5	6.3	50.7
	V	5	11.1	6.2	11.8	5.8	61.8
	VI	4	13.1	9.4	11.7	5.2	60.3
6 June	I	2	13.8	6.0	18.1	4.2	58.6
	II	3	14.7	9.8	19.2	6.1	50.1
	III	2	14.3	5.2	23.4	4.6	52.4
	IV	3	16.2	8.2	19.1	5.7	50.8
	V	4	11.1	6.9	17.1	6.1	55.9
	VI	4	16.5	7.5	12.2	5.8	58.1
8 June	I	0					
	II	1	12.0	7.1	13.3	6.0	61.9
	III	2	10.1	6.0	26.5	3.3	51.3
	IV	1	13.5	8.4	19.4	5.6	52.8
	V	2	14.8	7.8	19.2	6.0	51.8
	VI	4	17.3	8.6	11.0	5.2	57.4
10 June	I	0					
	II	1	13.1	7.2	12.0	5.9	59.0
	III	0					
	IV	0					
	V	1	9.5	6.0	19.9	5.3	59.0
	VI	4	15.5	5.6	10.8	5.3	62.6

* Day challenged.

dogs, group VI, was slightly increased with the maximum alteration occurring on the eighth post challenge day.

The mean values for the terminal sera are compared on a group basis in table 6. The group VI "terminal" values were determined by using the figures obtained for the sera from June 6 and 8. The maximum alteration occurred on these dates.

Table 6. Comparison of terminal serum protein alterations.

Group	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin
I	2	12.9	5.8	19.2	3.8	59.1
II	2	15.4	10.4	21.9	5.7	46.5
III	3	12.1	5.8	26.4	4.1	49.8
IV	3	15.8	8.2	19.2	5.5	51.0
V	5	10.1	6.5	17.0	5.1	59.0
VI	4	15.1	5.6	10.8	5.3	62.6

The per cent alteration of the average terminal serum protein fraction for each group has been compared in tables 7 and 8. The per cent alteration in table 7 was calculated using as the normal value for the fraction the average results obtained for the group from May 23 and May 31 sera. The values in table 8 were determined by using as the normal value the mean of the values for all the dogs in groups I through VI.

Table 7. Per cent alteration in the terminal serum proteins of each group using the May 23 and May 31 mean for the group as the base value.

Group	Gamma	Beta	Alpha 2	Alpha 1	Albumin
I	- 3 %	+ 16%	+ 73%	- 12%	- 12%
II	+ 3	+ 82	+146	- 16	- 25
III	-17	+ 53	+167	- 36	- 22
IV	+ 5	+ 82	+ 83	0	- 17
V	-20	+ 25	+118	- 6	- 11
VI	+19	+ 10	+ 29	0	- 10

Table 8. Per cent alteration in the terminal serum proteins of each group using the May 23 and May 31 mean for all dogs as the base value.

Group	Gamma	Beta	Alpha 2	Alpha 1	Albumin
I	- 9%	0%	+ 106%	- 31%	- 8%
II	+ 8	+ 79	+ 135	+ 4	- 28
III	-15	0	+ 184	- 25	- 22
IV	+11	+ 41	+ 106	0	- 20
V	-29	+ 12	+ 83	- 7	- 8
VI	+19	+ 38	+ 25	0	- 10

The most consistent and most marked alteration occurred in the elevation of alpha 2 globulin in groups I through V. A consistently small decrease in albumin was also noted in these groups. The immune group VI showed a small elevation of gamma and alpha 2 globulins and a small decrease in albumin.

Alterations in beta and alpha 1 globulins were not considered significant, as the variations were not consistent and the percentage of these two fractions in the serum was small, thus a slight change resulted in a large per cent alteration.

The mean values of all dogs in groups I through V are compared on a day basis in table 9. The mean values for the sixth and eighth of June for group VI are presented for comparison. The increase in alpha 2 globulin on June 10 resulted from the inclusion of dog number 38 which did not die until June 14 in group II. The alpha 2 value for the other dog included in the date which died on June 10 is 19.9 per cent. A slightly increased gamma globulin in the immune group was demonstrated.

The results of the statistical analysis of the gamma, beta and alpha 2 fractions in these sera are given in table 10.

Table 9. Comparison on a day basis of the mean serum protein values of all dogs in groups I through V

Date	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin
May 23	16	14.5	5.3	8.8	5.4	65.8
*May 31	15	14.0	6.4	9.7	5.5	62.4
June 2	16	14.8	7.8	11.3	5.8	60.0
June 4	16	14.8	7.4	14.4	5.8	57.4
June 6	14	14.5	7.4	19.2	5.5	53.4
June 8	7	12.2	6.8	21.0	4.7	54.2
June 10	2	11.3	6.6	15.9	5.6	60.3
**	4	16.9	8.0	11.6	5.5	57.7

* Day challenged.

** The group VI mean values for June 6 and 8.

Table 10. Results of a statistical analysis designed to show the significance of the variation in the gamma, beta and alpha 2 serum protein fractions in groups I-V.

Globulin fraction	Source of variation	Theoretical 1% variance ratio	Variance ratio in experiment	Significance
Gamma	Between dogs	1.86	7.33	***
	Between dates	2.76	0.70	NS
	Between dogs and dates	1.59	1.93	**
Beta	Between dogs	1.86	2.78	**
	Between dates	2.76	22.07	***
	Between dogs and dates	1.59	2.17	**
Alpha 2	Between dogs	1.86	15.65	***
	Between dates	2.76	51.67	***
	Between dogs and dates	1.59	2.89	***

NS Not significant.

* Probability of resulting from chance is 1 in 20.

** Probability of resulting from chance is 1 in 100.

*** Probability of resulting from chance is 1 in 1,000.

Glycoprotein, Groups I-VI

The mean serum glycoprotein values within each group are compared in table 11. The primary alteration in serum glycoprotein was an elevation of the alpha 2 fraction. A slight elevation occurred on the second post challenge day and a rapid, marked increase occurred on subsequent days.

The average serum glycoprotein values between groups are presented on a day basis in table 12. Here again the main alteration was in alpha 2 globulin, with the elevation commencing on the second post challenge day.

The per cent alteration in the terminal serum glycoprotein values are compared in tables 13 and 14. The per cent alteration in table 13 was determined by using the average of the May 23 and May 31 values as normals for the group. The figures in table 14 were obtained by using the average of the May 23 and 31 values for all the dogs in groups I through VI. The per cent alteration in the tryptophane-rich pre-albumin was not determined. This fraction exists in such a small amount that its determination was not considered sufficiently accurate to permit analysis for alterations in quantity.

The mean values for all dogs in groups I through V are compared with the average values for the fourth and sixth of June for group VI on a day basis in table 15. A marked increase in alpha 2 globulin was seen.

The statistical analysis of the glycoprotein fraction values is presented in table 16.

Statistical analysis to determine the correlation between gamma globulin and glycoprotein, beta globulin and glycoprotein and alpha 2 globulin and glycoprotein showed no correlation for the gamma and beta fractions. Between alpha 2 globulin and glycoprotein there was a 0.61 correlation on May 23 but none thereafter.

Table 11. Comparison of serum glycoprotein variations within groups.

Group	Date	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin
I	23 May	1	19.9	23.2	23.2	18.1	2.6
	*31	1	17.7	15.7	27.7	37.0	1.8
	2 June	1	20.5	11.8	45.1	20.4	2.1
	4	2	19.3	13.9	45.0	19.8	1.8
	6	2	17.0	19.6	48.7	11.9	2.7
	Terminal	2	17.0	19.6	48.7	11.9	2.7
II	23 May	3	19.2	15.7	28.3	33.6	3.1
	*31	3	20.2	16.1	39.7	20.8	3.4
	2 June	3	19.5	17.8	35.0	19.9	4.5
	4	3	18.4	20.8	43.9	22.7	3.2
	6	3	15.1	15.3	43.4	23.2	2.9
	8	1	23.6	17.0	35.8	20.8	2.8
	10	1	25.4	25.7	30.5	16.1	2.4
	Terminal	2	12.8	14.0	43.3	25.5	2.9
III	23 May	3	19.4	17.4	38.4	21.1	3.6
	*31	3	25.2	17.4	29.5	22.2	5.7
	2 June	3	23.0	15.6	32.7	24.0	4.7
	4	3	22.3	17.4	40.5	17.6	2.5
	6	3	20.5	11.0	48.3	17.8	3.0
	Terminal	3	20.5	11.0	48.3	17.8	3.0
IV	23 May	3	21.6	17.7	34.9	21.5	4.3
	*31	3	19.3	18.3	21.9	34.1	6.3
	2 June	3	24.7	15.3	25.5	30.7	3.8
	4	3	18.6	19.4	33.9	23.9	4.1
	6	3	17.9	18.3	35.9	23.7	4.2
	8	1	17.2	18.5	42.9	20.0	1.5
	Terminal	3	17.6	16.8	37.2	23.9	4.4
V	23 May	5	21.1	17.5	29.2	29.4	2.8
	*31	5	25.5	14.4	30.3	24.8	5.0
	2 June	5	19.8	18.0	33.7	22.9	5.5
	4	5	16.3	16.7	34.2	25.5	4.6
	6	4	13.6	15.5	49.3	19.6	1.8
	8	2	13.0	20.4	42.0	23.1	1.3
	10	1	12.9	17.3	50.5	16.6	1.2
	Terminal	5	13.3	16.7	49.5	16.3	4.1
VI	*31 May	4	20.0	21.5	26.8	28.8	3.1
	2 June	3	23.5	18.2	27.2	28.4	2.6
	4	4	17.8	20.7	31.3	28.4	1.8
	6	3	21.3	15.2	31.6	25.3	1.6
	8	4	18.1	21.1	28.6	30.7	1.5
	10	4	19.5	22.5	33.0	23.5	3.0

* Day challenged.

Table 12. Comparison of serum protein alterations between groups on a day basis.

Date	Group	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin
23 May	I	1	19.9	23.2	23.2	18.1	2.6
	II	3	19.2	15.7	28.3	33.6	3.1
	III	3	19.4	17.4	38.4	21.1	3.6
	IV	3	21.6	17.7	34.9	21.5	4.3
	V	5	21.1	17.5	29.2	29.4	2.8
*31 May	I	1	17.7	15.7	27.7	37.0	1.8
	II	3	20.2	16.1	39.7	20.8	3.4
	III	3	25.2	17.4	29.5	22.2	5.7
	IV	3	19.3	18.3	21.9	34.1	6.3
	V	5	25.5	14.4	30.3	24.8	5.0
	VI	4	20.0	21.5	26.8	28.8	3.1
2 June	I	1	20.5	11.8	45.1	20.4	2.1
	II	3	19.5	17.8	35.0	19.9	4.5
	III	3	23.0	15.6	32.7	24.0	4.7
	IV	3	24.7	15.3	25.5	30.7	3.8
	V	5	19.8	18.0	33.7	22.9	5.5
	VI	3	23.5	18.2	27.2	28.4	2.6
4 June	I	2	19.3	13.9	45.0	19.8	1.8
	II	3	18.4	20.8	43.9	22.7	3.2
	III	3	22.3	17.4	40.5	17.6	2.5
	IV	3	18.6	19.4	33.9	23.9	4.1
	V	4	16.3	16.7	34.2	25.5	4.6
	VI	4	17.8	20.7	31.3	28.4	1.8
6 JUne	I	2	17.0	19.6	48.7	11.9	2.7
	II	3	15.1	15.3	43.3	17.8	3.0
	III	3	20.5	11.0	48.3	17.8	3.0
	IV	3	17.9	18.3	35.9	23.7	4.2
	V	4	13.6	15.5	49.3	19.6	1.8
	VI	3	21.3	15.2	31.6	25.3	1.6
8 June	I	0					
	II	1	23.6	17.0	35.8	20.8	2.8
	III	0					
	IV	1	17.2	18.5	42.9	20.0	1.5
	V	2	13.0	20.4	42.0	23.1	1.3
	VI	4	18.1	21.1	28.1	30.7	1.5
10 June	I	0					
	II	1	25.4	25.7	30.5	16.1	2.4
	III	0					
	IV	1	17.2	18.5	42.9	20.0	1.5
	V	1	12.3	17.3	50.5	16.6	1.2
	VI	4	19.5	22.5	33.0	23.5	3.0

* Day challenged.

Table 13. Comparison of per cent alteration in terminal serum glycoprotein values between groups using the May 23 and May 31 mean for each group as the base figure.

Group	Gamma	Beta	Alpha 2	Alpha 1
I	- 10%	+ 1%	+ 92%	- 57%
II	- 35	- 12	+ 27	- 6
III	- 8	- 37	+ 43	- 18
IV	- 14	- 7	+ 31	- 14
V	- 43	+ 5	+ 67	- 40
VI	- 1	- 16	+ 12	- 3

Table 14. Comparison of per cent alteration in terminal glycoprotein values between groups using the May 23 and May 31 mean for all dogs as the base value.

Group	Gamma	Beta	Alpha 2	Alpha 1
I	- 21%	+ 17%	+ 54%	- 53%
II	- 41	- 16	+ 37	0
III	- 5	- 34	+ 53	- 30
IV	- 19	+ 0.6	+ 18	- 6
V	- 40	0	+ 57	- 36
VI	- 8	+ 8	- 5	+ 10

Table 15. Comparison on a day basis of the mean glycoprotein values of all dogs in groups I-V.

Date	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1
May 23	16	20.5	16.7	32.6	26.2
* 31	15	22.7	16.7	30.7	24.9
June 2	15	21.2	16.8	31.7	25.0
4	16	18.6	17.8	36.8	22.9
6	15	16.6	15.7	45.8	19.7
8	4	16.7	21.2	40.7	21.8
10	2	19.8	21.5	40.5	16.4
**	4	18.6	20.5	31.4	27.2

* Day challenged

** June 4-June 6 mean values for group VI.

Table 16. Results of a statistical analysis designed to show the significance of the variation in the gamma, beta, alpha 2 and alpha 1 serum glycoprotein fractions.

Serum fraction	Source of variation	Theoretical 1% variance ratio	Variance ratio in experiment	Significance
Gamma	Between dogs	1.92	1.24	NS
	Between dates	2.56	6.18	***
Beta	Between dogs	1.92	3.23	**
	Between dates	2.56	1.09	NS
Alpha 2	Between dogs	1.92	4.47	***
	Between dates	2.56	10.45	***
Alpha 1	Between dogs	1.92	2.92	**
	Between dates	2.56	1.63	NS

NS Not significant.
** Probability of resulting from chance is 1 in 100.
*** Probability of resulting from chance is 1 in 1,000.

Leukocyte Counts, Groups I-VI

The mean total leukocyte counts within each group are compared on a day basis in table 17. In table 18 the mean leukocyte counts for groups I through V are compared on a day basis with the mean values for group VI. The white blood cell counts in both these tables showed little difference between the susceptible and immune dogs through June 6. Despite the apparent close correlation in number of leukocytes on June 6, a decrease in leukocytes in all groups began about June 4. After June 6 the number of leukocytes in the susceptible dogs continued to decrease while the number in the immune dogs increased.

Table 17. Comparison of mean total leukocyte counts between groups on a day basis.

Group					Challenged					
		23 May	27 May	29 May	31 May	2 June	4 June	6 June	8 June	10 June
I	*	2	2	1	2	2	2	2		
	WBC	10,750	11,550	10,300	10,800	10,700	9,080	7,510		
II	*	3	2	3	2	3	3	3	1	1
	WBC	11,300	12,250	10,513	8,140	10,123	14,066	8,030	8,020	6,540
III	*	3	3	3	3	3	3	3		
	WBC	13,466	12,200	14,800	11,266	12,133	11,356	6,990		
IV	*	3	3	3	3	3	3	3		
	WBC	10,686	10,126	11,056	10,466	12,933	9,800	7,000		
V	*	5	5	5	5	5	5	5	2	
	WBC	11,400	13,560	12,160	10,260	12,960	12,940	10,766	4,585	
VI	*	4	4	4	4	4	4	4	4	3
	WBC	11,750	10,360	10,140	9,274	10,900	9,526	8,436	12,260	12,967

* Number of dogs on which the value stated is based.

Table 18. Comparison on a day basis of the mean total leukocyte counts of groups I through VI with the mean values for group VI.

Date	No. of dogs	Mean for Groups I-V	Mean for Group VI	No. of dogs
May 23	16	11,535	11,750	4
27	14	12,156	10,360	4
29	15	12,014	10,140	4
* 31	15	10,292	9,274	4
June 2	16	11,982	10,900	4
4	16	11,783	9,526	4
6	15	8,459	8,436	4
8	3	5,862	12,260	4
10	2	10,620	12,967	3

* Day challenged.

Statistical analysis of the white blood cell counts showed no significant change between dogs, but a 0.05 significance between dates (probability of resulting from chance 1 in 1,000).

Temperatures, Groups I-VI

The average daily temperature within each group is compared in table 19. In table 20 the mean daily temperatures for groups I through V are compared with the mean daily temperatures for group VI. In both these tables the temperatures gradually increase in all groups up to June 4. Subsequently the temperatures for the susceptible dogs continues to increase while the temperatures for the immune dogs returned to normal.

Statistical analysis of the temperatures showed a significance of 0.01 (probability of resulting from chance 1 in 100) between dogs and a 0.001 (1 in 1,000) between dates.

Dogs in groups I through V died a natural death or were euthanatized when moribund. The dogs in these groups all showed signs of a severe central nervous system disturbance. At the time of necropsy, they were in good physical condition and showed no gross lesions. Histopathological examination was done on the following tissues: trachea, lung, stomach, small intestine, large intestine, kidney, bladder, spinal cord, restiform body, cerebellar peduncle, cerebellum, cerebral peduncle and liver. The only consistent lesion in groups I through V was a slight interstitial pneumonia. No inclusion bodies were seen with hematoxylin and eosin stain or in the sections stained with Shorr's S-3 stain.

Table 19. Comparison of the mean daily temperatures for groups I through V with the mean temperature for group VI.

Date	No. of dogs	Mean temperature for groups I-V	Mean temperature for group VI	No. of dogs
May 30	15	102.5	102.0	3
* 31				
June 1	15	102.3	102.3	4
2	16	102.3	101.3	3
3	16	103.6	102.5	4
4	16	103.9	103.0	3
5	16	104.0	101.4	4
6	16	103.0	100.7	4
7	6	103.2	101.6	4
8	4	102.8	101.9	4
9	3	102.9	101.6	4
10	2	103.8	101.8	4

* Day of challenge.

The relationship between leukocyte counts, temperature, alpha 2 globulin and glycoprotein for groups I through V is shown in figure 1, and for group VI in figure 2.

Table 20. Comparison of the mean daily temperatures between groups.

Groups		30 May	31 May	1 June	2 June	3 June	4 June	5 June	6 June	7 June	8 June	9 June	10 June
I	*	2		2	2	2	2	2	2	2			
	Temp	102.7		102.6	103.4	104.2	104.4	104.5	103.5	104.4			
II	*	3		3	3	3	3	3	3	1	1	1	1
	Temp	102.5		101.7	102.0	103.5	104.1	103.7	103.1	103.0	103.2	103.2	103.8
III	*	3		3	3	3	3	3	3				
	Temp	102.4		102.4	101.6	102.7	103.6	104.3	104.2				
IV	*	3		2	3	3	3	3	3	1	1		
	Temp	102.8		102.5	102.2	103.7	103.7	103.7	102.7	103.6	103.0		
V	*	4		5	5	5	5	5	5	2	2	2	1
	Temp	102.4		102.4	102.4	103.8	103.9	104.0	103.1	101.9	102.0	102.7	103.8
VI	*	3		4	3	4	3	4	4	4	4	4	4
	Temp	102.0		102.3	101.3	102.5	103.0	101.4	100.7	101.6	101.9	101.6	101.8

* Number of dogs on which the mean value is based.

Table 21. The comparison of mean serum protein values of groups VII and VIII on a day basis.

Date	Group VII						Group VIII					
	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin	No. of dogs	Gamma	Beta	Alpha 2	Alpha 1	Albumin
25 Apr.	4	19.3	11.0	13.0	4.2	52.4	4	19.6	8.8	10.9	3.9	56.4
* 11 May	4	18.8	12.4	17.4	4.8	40.0	2	19.4	14.0	19.5	4.2	50.2
16	4	20.2	12.4	19.1	5.2	42.7	3	15.1	8.5	23.4	3.3	48.8
18	4	21.0	11.0	17.6	4.7	44.8	3	14.9	9.7	19.4	3.8	52.6
20	4	20.0	9.8	20.8	5.2	44.5	3	17.7	11.2	20.6	3.8	50.0
22	3	22.0	9.7	20.4	5.3	40.8	3	13.8	7.5	33.3	4.5	41.3
24	2	17.5	11.8	20.1	5.2	47.9	2	14.9	5.9	27.4	3.6	43.6
26							2	13.2	7.8	30.2	3.8	45.2
30							1	15.6	10.1	42.2	4.5	27.4
4 June							1	11.9	12.7	40.0	3.6	32.7
7	4	28.1	11.2	16.7	4.3	40.6						

* Day challenged.

Table 22. Comparison of per cent alteration in groups VII and VIII using the mean of the 25 April and 11 May figures for the group as the base value.

Date	Group VII					Group VIII				
	Gamma	Beta	Alpha 2	Alpha 1	Albumin	Gamma	Beta	Alpha 2	Alpha 1	Albumin
16 May	+ 6.3	+ 6.0	+ 25.0	+ 15.5	- 7.6	- 22.6	- 24.1	+ 53.0	- 17.5	- 8.4
18	+ 10.5	- 6.0	+ 15.8	+ 4.4	- 3.1	- 23.6	- 20.5	+ 27.6	- 5.0	- 1.4
20	+ 5.3	-16.3	+ 36.8	+ 15.6	- 3.7	- 9.8	- 8.2	+ 35.5	- 5.0	- 6.4
22	+ 15.8	-17.1	+ 34.2	+ 17.8	- 13.3	- 29.3	- 29.5	+119.0	+ 12.5	- 22.5
24	- 7.9	+ 0.8	+ 32.2	+ 15.6	+ 3.7	- 23.6	- 47.3	+ 80.3	- 10.0	- 18.2
26						- 32.3	- 34.1	+ 98.7	- 5.0	- 15.2
30						- 20.0	- 17.2	+177.6	+ 12.5	- 55.0
4 June						- 39.0	+ 4.1	+163.2	- 10.0	- 38.6
7	+ 45.6	- 4.3	+ 9.7	- 4.5	- 12.1					
Terminal						- 30.8	- 21.3	+134.2	- 2.5	- 29.5

For the number of dogs on which these figures are based, see table 21.

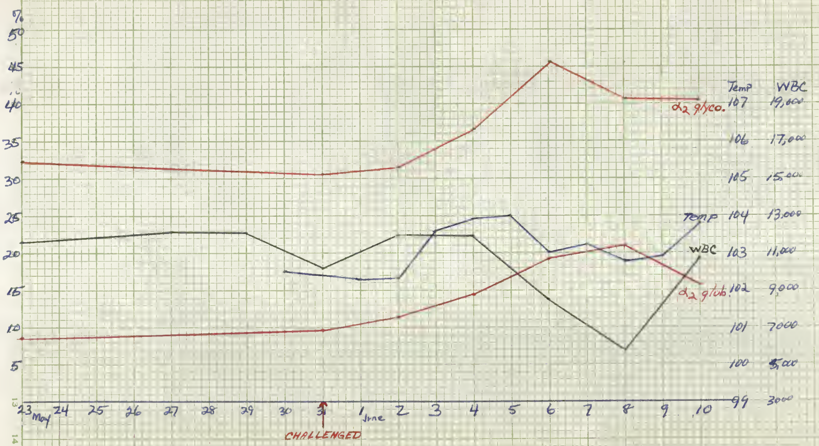

Fig. 1. The relationship between serum alpha 2 globulin, serum alpha 2 glycoprotein, leukocyte counts and temperatures using the average values for all dogs in groups I-V.

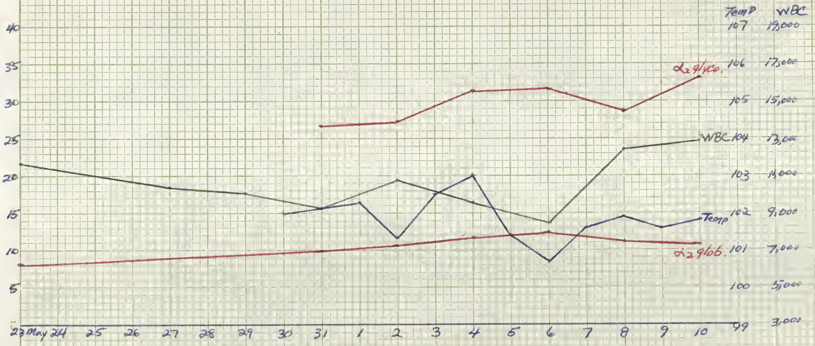

Fig. 2. The relationship between serum alpha 2 globulin, serum alpha 2 glycoprotein, leukocyte count and temperatures using the average values for all dogs in group VI.

Serum Proteins, Groups VII and VIII

The results of the electrophoretic separation of serum protein in groups VII and VIII are compared in table 21. The per cent alteration in groups VII and VIII is compared on a day basis in table 22. The base value used for determining per cent change was derived by averaging the April 25 and May 11 values for each fraction of groups VII and VIII. The changes noted in tables 21 and 22 were similar to the alterations that occurred in groups I through VI. The only difference was a greater increase in gamma globulin in the group that survived--group VII.

Leukocyte Counts, Groups VII and VIII

The total leukocyte counts for groups VII and VIII are compared on a day basis in table 23. The leukocyte counts in both groups were similarly elevated.

Table 23. Comparison of the mean total leukocyte counts for groups VII and VIII.

	Group VII		Group VIII	
Date	No. of dogs	WBC	No. of dogs	WBC
Apr. 25	4	16,300	4	16,950
May 2	4	13,267	4	15,250
* 11	4	13,730	4	18,025
16	4	21,025	4	23,475
18	4	20,875	4	17,247
20	4	19,100	4	16,525
22	3	18,400	4	17,100
June 7	2	15,100		

* Day challenged.

Temperatures, Groups VII and VIII

The daily temperatures for groups VII and VIII are compared in table 24. The temperatures in both groups were similar.

Table 24. Comparison of the mean daily temperatures for groups VII and VIII.

Date	Group VII		Group VIII	
	No. of dogs	Temperature	No. of dogs	Temperature
May 12	4	102.0	4	102.6
13	4	103.0	4	102.6
14	4	103.0	4	102.6
15	4	102.2	4	102.4
16	4	101.5	4	102.6
17	4	102.1	4	102.8
18	4	102.1	4	102.6
19	4	102.0	4	103.2
20	4	102.0	4	103.0
21	4	102.6	4	102.3
22	4	102.0	3	103.8

Group VIII dogs had been gradually becoming emaciated for about 2 weeks prior to death. One dog had chorea and the others had convulsions prior to euthanasia. At necropsy all the dogs were in fair to poor physical condition. Two had scaling, flaking skin, exudate in the eyes and cloudy mucous in the nostrils. All the dogs had some thick mucous in the trachea, and the ventral portions of all lobes of the lung showed red to grey consolidation. Microscopically the most consistent lesion was a suppurative bronchopneumonia.

DISCUSSION

The primary alteration demonstrated by use of paper electrophoresis in the serum from dogs with acute distemper was a marked increase in alpha 2 globulin, decrease in albumin and increase in alpha 2 glycoprotein. Since it has been shown that alpha 2 globulin was also increased in non-infectious inflammatory conditions (Gjeasing and Chanutin, 1946), rabies (Chabaud, 1955), Babesia canis infection (Polson and Malherbe, 1952), acute hepatic degeneration (Archibald and Vesselinovitch, 1957), alpha 2 hyperglobulinemia must be considered a non-specific reaction resulting from the effect of stress on the animal.

The increased alpha 2 glycoprotein, in view of the many agents producing an increase of this fraction in humans and increases in total glycoprotein in animals, has also been interpreted as a non-specific reaction.

Selye (1950) has interpreted the dysproteinemia, albumin decrease and alpha globulin increase observed in these dogs as a reaction to stress. During the acute stage of stress or initially following a stressful event, the adrenal cortical response has been found to produce a temporary elevation of alpha globulin equivalent to that obtained by injecting cortisone (Boschel et al., 1955; Kushner et al., 1956). The major elevation of alpha 2 globulin which occurs during the alarm and exhaustive stages has been ascribed to an increase in protein catabolism in which albumin is more readily metabolized than globulins (Selye, 1950), a rapid synthesis of alpha 2 globulin to replace the albumin (Wuhrmann and Wunderly, 1960, p. 351) and to tissue injury and destruction. The extent of this shift in albumin and globulin is stated to be dependent on the ability of the animal to react (Wuhrmann and Wunderly, 1960, p. 350).

The same causes have been ascribed to an increase in glycoprotein (Kushner et al., 1956).

The relationship between temperature, leukocyte count, serum alpha 2 globulin and glycoprotein for groups I through V shown in figure 1 was interpreted as two reactions. The elevation in alpha 2 globulin, alpha 2 glycoprotein and leukocyte count that occurred between May 31 and June 2 resulted from challenging procedures. The subsequent elevation was interpreted as showing the response of dogs to the stress produced by the multiplication of the virulent virus. The temperatures and leukocyte counts were used to indicate the degree of the reaction by the animal. The severity of the stress was manifested by the death of the dogs.

The increase in alpha 2 globulin, alpha 2 glycoprotein and leukocyte count in figure 2 between May 31 and June 2 was again due to the challenging procedures. The subsequent increase in alpha 2 globulin and glycoprotein and temperature and decrease in leukocyte count has been interpreted as stress produced by multiplication of the virus in the "immune" dogs. The dogs returned to normal after this boostering effect produced by the virulent virus.

Group VIII dogs showed the same response in alpha 2 globulin. The continually high leukocyte count and increased temperature has been attributed to the respiratory infection observed prior to and following challenge and at necropsy.

The small degree of significance in the statistical analysis of the leukocyte counts for groups I through V was thought to result from the fact that only those days for which figures were available for a majority of the dogs were evaluated. The counts on and after June 6, when the most pronounced alteration occurred, were excluded from the analysis.

The 0.61 correlation between alpha 2 globulin and glycoprotein that occurred on May 23, and the absence of correlation thereafter indicated that these two fractions rose independently of each other. However, more evidence should be obtained concerning this relationship.

The increase in gamma globulin in groups VI and VII was thought to result from an increased antibody titer produced by the virulent virus.

SUMMARY

Electrophoretic studies of the serum protein and glycoprotein levels were performed on multiple serum samples obtained from twehty susceptible and eight immune dogs inoculated intracerebrally with the Snyder Hill strain of distemper virus. A significant increase in alpha 2 globulin and alpha 2 glycoprotein was observed in the susceptible dogs. The relationship of increased alpha 2 globulin and alpha 2 glycoprotein to increasing temperature and decreasing leukocyte count was shown. The increase of these two fractions was interpreted as a non-specific response to the adverse effects of the virus on the host.

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ELECTROPHORETIC STUDIES OF SERUM PROTEINS AND GLYCOPROTEINS IN ACUTE CANINE DISTEMPER

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Serum protein, serum glycoprotein, temperature and leukocyte alterations in dogs experimentally infected with canine distemper were determined and compared.

The largest portion of the material presented was obtained from 6 groups of dogs. Group I contained 2 dogs, group II 3 dogs, group III 3 dogs and group IV 3 dogs. Groups I through IV were vaccinated with an attenuated live virus (Cytogen) 5 through 2 days respectively prior to challenge. Group V consisted of 5 dogs that were not vaccinated and group VI contained 4 immune dogs. All dogs were challenged intracerebrally with the Snyder Hill strain of distemper virus. Prevaccination sera were screened for distemper antibodies at 1:20 and 1:40 dilutions using the serum neutralization test in 7 day embryonated eggs. Using a Coulter Counter leukocyte counts were performed on all dogs before challenging, on the day of challenge and every other day thereafter until the dogs died. Blood for serum was collected when the blood for leukocyte counts was drawn. Temperatures were taken the day prior to challenging and daily thereafter. All dogs were necropsied and blocks of tissue were preserved in formalin. Histopathological examination showed only a slight interstitial pneumonia in groups I through V.

Serum protein separations were performed on a Spinco electrophoresis apparatus using a veronal buffer at a pH of 8.6, 0.006 ml. of serum and 15 milliamps per cell for 5 hours. The strips were stained with an alcoholic bromphenol blue dye and scanned on a Spinco Analytrol. Serum glycoprotein separations were performed using the same instrument, 0.03 ml. of serum and 20 milliamps per cell for 6 hours. These strips were stained with periodic Schiff's reagent.

Serum protein, glycoprotein, leukocyte and temperature alterations between groups were compared. The time of vaccination produced no apparent effect on the alterations observed. The primary change in terminal serum protein in groups I through V was an increase in alpha 2 globulin ranging from 83 per cent for group V to 184 per cent for group III. Group VI showed a 25 per cent increase. The main alteration in terminal serum glycoprotein was an increase in the alpha 2 fraction ranging from 18 per cent in group IV to 54 per cent in group I. Group VI showed a 5 per cent decrease in this fraction. The relationship between leukocyte count, temperature and serum alpha 2 globulin and glycoprotein was shown. As the temperature increased and leukocyte count decreased, the alpha 2 fractions increased.

The same examinations were performed on 4 susceptible and 4 immune dogs from another experiment. Similar serum alpha 2 globulin and glycoprotein alterations were observed. Due to the presence of a bronchopneumonia in these dogs, the relationship between temperature, leukocyte count and alpha 2 globulin and glycoprotein as seen in groups I through V was not present.